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Use of bacteriophages as a therapy for bacterial infection has been attempted over the last century. Such an endeavor requires the elucidation of basic aspects of the host–virus interactions and the resistance mechanisms of the host. Two recently developed bacterial collections now enable a genomewide search of the genetic interactions between *Escherichia coli* and bacteriophages. We have screened >85% of the *E. coli* genes for their ability to inhibit growth of T7 phage and >90% of the host genes for their ability to be used by the virus. In addition to identifying all of the known interactions, several other interactions have been identified. *E. coli* CMP kinase is essential for T7 growth, whereas overexpression of the *E. coli* uridine/cytidine kinase inhibits T7 growth. Mutations in any one of nine genes that encode enzymes for the synthesis of the *E. coli* lipopolysaccharide receptor for T7 adsorption leads to T7 resistance. Selection of T7 phage that can recognize these altered receptors has enabled the construction of phage to which the host is 100-fold less resistant.

host–virus interactions | T7 receptor | ASKA library | Keio collection | phage therapy

Bacteriophages have devised numerous strategies for growth in their bacterial host. In some instances they encode inhibitors of host proteins whose expression shortly after infection halt processes essential for host survival, substituting instead their own counterpart for phage growth. In other instances there is an acquisition of one or more host functions, thus minimizing the phage genome size. The identity of these phage–host relationships has, in the past, arisen largely from multiple independent studies. A systemwide approach for identifying these relationships would be valuable in that the ingenuity of the phage in acquiring host functions leads to unexpected, but extremely interesting, biochemical and genetic strategies. An understanding of these phage–host relationships at the molecular level could, in turn, lead to the construction of phages that depend less on certain properties of the host and consequently would be more virulent. Likewise, the identification of host proteins that hinder phage infection and growth would be instructive in designing phages that could overcome them. Such phages deplete their host with minimal acquired resistance and may thus serve as an attractive alternative to antibiotic treatment. It would be virtually impossible to isolate phages in nature to which no bacterial resistance readily evolves. Such phages deplete their host and thus eliminate their sole mechanism of propagation.

Bacteriophage T7 and its host, *Escherichia coli*, provide a model for systematically studying host–virus interactions. Their genetics have been studied extensively, and the functions of >50% of the 56 genes of T7 and 4,453 genes of *E. coli* have been elucidated. The recent development of two *E. coli* collections has enabled the systematic search for interactions between *E. coli* and T7 phage. One, the "Keio collection" (1) contains single-gene knockout mutants of all of the nonessential genes in *E. coli* K-12. The other, the "ASKA library" (2) contains *E. coli* cells expressing most of the *E. coli* genes from cloned plasmids.

A single T7 phage produces >100 progeny after infection of *E. coli*. The phage inhibits some *E. coli* proteins that interfere with its replication. For example, T7 produces a protein, gp0.3, that binds to and inhibits the *E. coli* type I restriction system (3). Other examples include the inhibition of the dGTP triphosphatase by the phage gp1.2 (4, 5) and the inhibition of the *E. coli* RNA polymerase by gp2 and gp0.7 (6–8). In this article we use the ASKA library to carry out a systematic screen to identify other *E. coli* genes that interfere with T7 growth.

A screen for genes that are essential for the intracellular growth of T7 was carried out by Chamberlin (9). One class of genes that he found, designated *tsnC*, consists of mutants in the *trxA* gene (10); the product of this gene, thioredoxin, acts as the processivity factor for T7 DNA polymerase (11). In this earlier study other essential genes may well have been missing in the mutant population because of nonrandom distribution of the induced mutations and the different growth characteristics of the mutated bacteria. In addition, Chamberlin chose not to characterize the *tsnA* group of genes that are essential for adsorption of T7 to the cell surface of *E. coli*. Identification of the biochemical defects in those mutants identified in this early screen was made difficult by the fact that proteins such as thioredoxin had not yet been mapped to a specific gene. A thorough and complete systematic screen covering virtually the entire nonessential *E. coli* genes has now become possible because of the development of the Keio collection (1).

Using the ASKA library and the Keio collection (1, 2), we have carried out two genetic screens, one for finding host genes that inhibit T7 growth and another for identifying host genes that are required for T7 growth. In addition to finding all of the expected genes in our screens, we have identified several genes that were not known to be involved in T7 growth. We have used our findings to construct, by genetic selections, T7 phage that are more resistant to mutations in the host than is WT T7 phage.

Results and Discussion

Screen for *E. coli* Genes that Interfere with T7 Phage Growth. We used the ASKA library to screen for host genes interfering with T7 growth (Fig. 1). The ASKA library contains 85% of the *E. coli* ORFs under the isopropyl β-D-thiogalactoside (IPTG)-inducible *tac* promoter on high-copy plasmids (2). We plated the ASKA library on agar that contains T7 phage and IPTG, as described in *Materials and Methods*. Three different T7 phages, D104/LG37, LG30, and HS33, were used for three independent screens; each was deleted for a nonessential region of T7 (see *Materials and Methods* for genes deleted). Using these phages enables the identification of genes in the host that can be overcome by phage-encoded genes.

After overnight incubation, surviving colonies were identified. We assume these colonies are resistant to T7 because the gene encoded by the plasmid confers T7 resistance. Another possibility is that these colonies acquired a secondary mutation that renders them resistant. To distinguish between these two possibilities,

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The authors declare no conflict of interest.

Abbreviations: IPTG, isopropyl β-D-thiogalactoside; EOP, efficiency of plating; MOI, multiplicity of infection.

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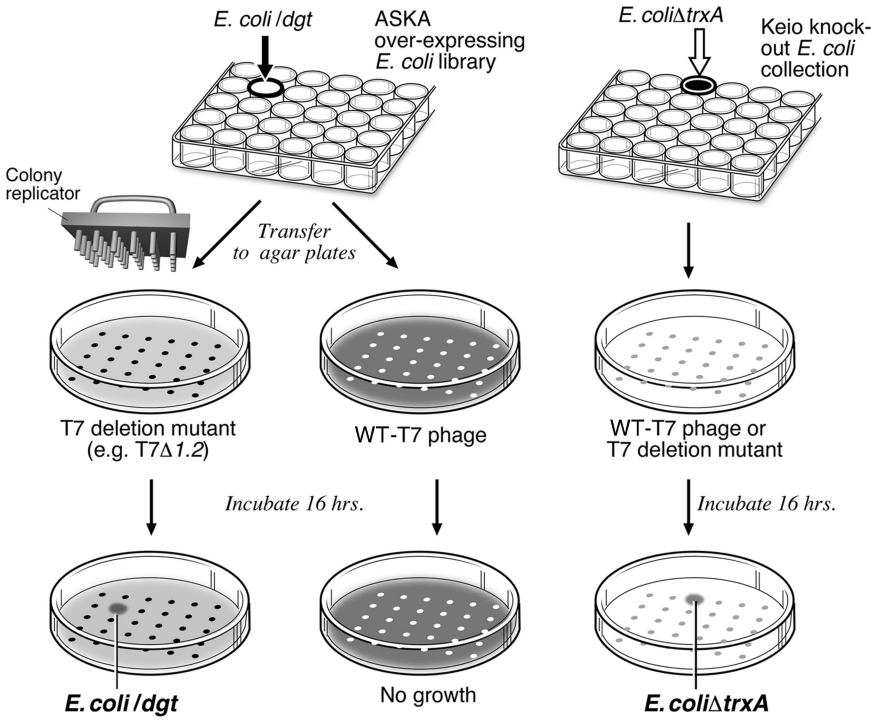

Fig. 1. Schematic presentation of the screening procedure. Cells were transferred from the *E. coli* collections onto agar plates layered with T7 phage and grown overnight. (*Left*) The identification of *E. coli* overexpressing the dGTP triphosphatase (*E. coli*/*dgt*). *dgt* overexpression inhibits T7 growth, but can be overcome by the activity of gp1.2 (5). A phage deleted for gene *1.2* would identify *E. coli*/dgt, whereas a screen with the WT T7 would not. (*Right*) A similar procedure for screening the Keio collection. Use of deletion T7 mutants in this procedure can lead to identification of genes in *E. coli* that are homologues of those deleted in the T7 phage.

plasmids from the original plates that had given rise to these surviving colonies were extracted and retransformed into a fresh stock of cells. After this procedure, the phenotype was tested again by plating on T7-containing agar. We have identified four genes that when overexpressed inhibit T7 growth on *E. coli*. The efficiency of plating (EOP) of the T7 deletion mutants on these strains as compared with a control is indicated in Table 1.

As expected, the two genes that are known to inhibit T7 growth when overexpressed, *hsdR* and *dgt* (4, 5, 12), were identified when T7 encompassing deletions in genes *0.3* and *1.2*, respectively, was used (Table 1). *hsdR* constitutes the restriction subunit of EcoK1, which, as mentioned above, is inhibited by gp0.3. *dgt* encodes a nucleotide triphosphatase that hydrolyzes dGTP. The dGTPase is inhibited by T7 gp1.2, and T7Δ*1.2* cannot grow on a *dgt*-overexpressing strain (*optA1*) (4, 5). These two genes were not identified when using T7 lacking genes other than *0.3* and *1.2*. These results validate the screening methodology designed to identify genes that have a detrimental influence on T7 growth.

The third gene identified in this screen was *rcsA*. *rcsA* encodes an activator of capsule synthesis genes. After expression of RcsA, colonies overproduce colanic acid, the major component of *E. coli* K-12 capsule (13, 14). Overproduction of the capsule is accompanied by a mucoid phenotype. The capsule of *E. coli* K1 is known to form a barrier to T7 infection (15); our finding extends these findings to *E. coli* K-12 whose capsule contains colanic acid (13). Overexpression of *rcsA* results in resistance to D104/LG37, LG30, HS33, and WT T7; presumably overproduction of the host capsule prevents phage adsorption. Even though the EOP on the host overexpressing *rcsA* was high (0.9), the plaques were significantly more turbid than the control. This observation suggests that only cells that express *rcsA* at an optimal level are resistant to T7 infection.

Table 1. Genes identified as inhibitors of T7 growth

Gene	Gene product	T7 gene that overcomes inhibition	EOP*
dgt	dGTP triphosphatase	*1.2*	$<10^{-2}$
hsdR	Subunit of the EcoKI restriction enzyme	*0.3*	0.06
rcsA	Activator of capsule synthesis genes	None	0.9†
udk	Uridine/cytidine kinase	*0.7*	$<10^{-2}$

*EOP of T7 D104/LG37 relative to its EOP on WT *E. coli*.
†Turbid plaques.

The fourth gene that interferes with T7 phage growth when overexpressed is the *udk* gene, a gene that encodes for uridine/cytidine kinase (16). The *udk* gene was identified in the screen by using D104/LG37 T7, which lacks genes *0.3*, *0.4*, *0.5*, *0.6A*, *0.6B*, *0.7*, *1.1*, *1.2*, *1.3*, and *1.4*. To identify the T7 gene that overcomes the effect of *udk* overexpression, we compared the ability of different T7 mutants to grow on *udk*-overexpressing colonies (Fig. 2). A phage encoding for a truncated gp0.7 (T7-JS62a) lyses *udk*-overexpressing cells significantly slower than it lyses control cells. WT T7 lyses *udk*-expressing cells only slightly slower than it lyses the control. Clearly, there is sufficient gp0.7 produced by the WT to inhibit the *udk* overexpression, but not to completely cure it. All of the other tested T7 deletion mutants, which express gp0.7 normally, lysed *E. coli* expressing *udk* as rapidly as did the WT T7 (data not shown). We therefore conclude that gp0.7 overcomes the effect of *udk* overexpression. Gp0.7 is a protein kinase that is known to phosphorylate *E. coli* RNA polymerase (17, 18), but the specific mechanism by which gp0.7 overcomes the inhibition of *udk* overexpression is unknown. We speculate that overexpression of *udk* interferes with the inhibition of the host RNA polymerase by T7. A T7 suppressor to the phenotype has been mapped to gene *2*, and

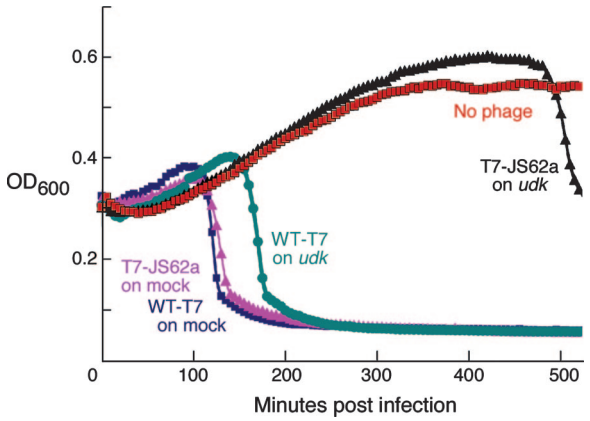

Fig. 2. Growth curves of *E. coli udk*- or mock-overexpressing cells infected with WT T7 or T7-JS62a. T7-JS62a contains a nonsense mutation in gene *0.7* at codon 126. *E. coli* cells harboring a plasmid encoding the *udk* gene or *thrS* gene (mock) were grown to midlog phase, induced, and infected by the designated T7 phage. OD_{600} measurements were taken every 5 min as described in *Materials and Methods*.

gp2, similarly to gp0.7, plays a role in host RNA polymerase inhibition.

Thus the genomewide screen not only identified all of the known genes that interfere with T7 phage growth but also identified two additional genes, *rcsA* and *udk*. T7 phage has evolved proteins that overcome the effect of three of these four genes. It is theoretically possible to allow T7 to overcome the effect of the gene activating capsule expression (*rcsA*) by introducing a gene product that hydrolyzes the colanic acid capsule. Indeed, a similar approach has been successfully demonstrated on *E. coli* K1 by using a T7 phage encoding an enzyme that hydrolyzes the K1 capsule (15).

Screen for *E. coli* Genes That Are Essential for T7 Phage Growth. To identify host genes that are required for T7 growth, we carried out a screen using the Keio collection (1) (Fig. 1). The collection was plated on an agar layer of T7 phage, and colonies that survived were further screened for the effect of the deleted genes on T7 growth. In this collection, ≈90% of *E. coli* genes could be individually deleted, indicating that under rich medium growth conditions they are nonessential. Three different T7 phage containing deletions in a number of T7 nonessential genes have been used for three independent screens. The use of T7 deletion mutants allows us to also detect host genes that are essential for T7 growth only when a T7 gene is lacking. For example, gene *1.3* encodes for a ligase that is nonessential for growing on WT host, because the phage can use the host functional ligase. However, gene *1.3* becomes essential for growing on a host in which ligase is not functional at the time of infection (19). Thus, using T7Δ*1.3* one would theoretically identify a host lacking the ligase gene. Practically, because ligase is essential for *E. coli*, it is not represented in the library, and therefore it would not be identified in our screen.

Table 2 summarizes the mutants that were identified after the screening procedure. We have validated the phenotype by measurement of the EOP on the identified clones and complementation of the replaced gene with a plasmid encoding for this gene. The genes identified can be classified into three groups: (*i*) *trxA*, (*ii*) *cmk*, and (*iii*) LPS biosynthesis genes. All bacteria deleted in these genes showed a significantly lower EOP as compared with the control bacteria. In addition, all of the identified deletion mutants were complemented by a plasmid encoding the respective deleted gene.

The *trxA* gene, as expected, was identified in this screen as being essential for T7 growth. The *trxA* gene encodes thioredoxin, the processivity factor for T7 DNA polymerase (11).

A second essential gene for T7 growth is *cmk*. *cmk* encodes for

Table 2. Genes identified as essential for T7 growth

Gene	Gene product*	EOP†	EOP after complementation§
trxA	Thioredoxin A	$<10^{-2}$	1
cmk	CMP/dCMP kinase	0.05‡	0.91
gmhA	LPS biosynthesis enzyme	$<10^{-2}$	0.86
gmhB	LPS biosynthesis enzyme	0.30‡	0.83
waaC	LPS biosynthesis enzyme	$<10^{-2}$	0.80
gmhD	LPS biosynthesis enzyme	$<10^{-2}$	0.16
gmhE	LPS biosynthesis enzyme	0.01	0.88
waaF	LPS biosynthesis enzyme	0.23‡	0.79
waaG	LPS biosynthesis enzyme	0.01	0.82
galU	LPS biosynthesis enzyme	0.29‡	0.82
waaR	LPS biosynthesis enzyme	$<10^{-2}$	0.79

*For detailed description of gene product, see Fig. 3.
†EOP of WT T7 relative to its EOP on WT *E. coli*.
‡Small/turbid plaques.
§EOP of WT T7 on indicated host harboring a plasmid, which encodes the deleted gene, relative to the EOP on WT *E. coli*.

CMP kinase that catalyzes the conversion of CMP and dCMP into CDP and dCDP, respectively, using ATP as the phosphate donor (20). This gene is not essential for *E. coli* but in its absence bacteria replicate at a slower rate (21). T7 phage, upon infection of *E. coli*, express an exonuclease (gp6) and an endonuclease (gp3) that hydrolyze the host chromosome to deoxyribonucleoside 5′-monophosphates. The resulting nucleotides are then presumably converted to the corresponding nucleoside diphosphates and triphosphates, the latter being the substrates for T7 DNA polymerase. Earlier studies have shown that >80% of the nucleotides found in T7 phage are derived from the breakdown of host DNA (22). The dependency of T7 on this salvage pathway most likely leads to a requirement for nucleoside 5′-monophosphate kinases for each of the four nucleosides. Other nucleoside monophosphate kinases (encoded by *adk*, *tmk*, *gmk*, *and pyrH*) are essential genes for *E. coli* and therefore would not arise in this screen. Precisely why *E. coli* also does not depend on CMP kinase is not clear but it may reflect the presence of other pathways that give rise to dCDP because cytosine nucleotides originate by amination of UTP, yet the conversion to deoxynucleotides by ribonucleotide reductase occurs only at the level of the nucleoside diphosphate (21).

The other genes identified to be essential for T7 infection encode for nine enzymes that are involved in the biosynthesis of the outer membrane LPS. It is known that LPS recognition by the T7 receptor is essential for phage adsorption to the host, but the exact sugar moiety to which the phage binds is not known (22). The core structure of *E. coli* K-12 LPS is depicted in cartoon form in Fig. 3, along with mutant LPS structures (chemotypes). The LPS core is composed of lipid A, which is embedded in the outer membrane and to which a backbone of Kdo, heptoses, and glucoses are attached. The genes *gmhA*, *gmhB*, *gmhD*, and *gmhE* encode for enzymes in the heptose biosynthesis pathway. Absence of any of these genes results in a type Re LPS, having only the Kdo sugars, as shown in Fig. 3 [an exception being *gmhB*, which has a partial Re phenotype (23) and may explain the different EOP measured on this strain compared with the other Re mutants]. Other genes that participate in enzymatic steps of the LPS core biosynthesis are: *waaA*, *waaC*, *waaF*, *waaG*, *galU*, *waaO*, *waaR*, and *waaK*, as depicted (24) [*galU* exhibits a leaky LPS phenotype (25), which may account for the differences in the EOPs between *galU* and *waaG*]. All of these genes except *waaA*, *waaO*, and *waaK* were identified in our screen. The *waaA* and *waaU* genes, catalyzing the addition of the Kdos and the terminal heptose, respectively, could not be identified because they are not represented in the Keio collection (1). The *waaO* was not identified in the screen and was shown to be not essential to T7 infection. The fact that addition of a single

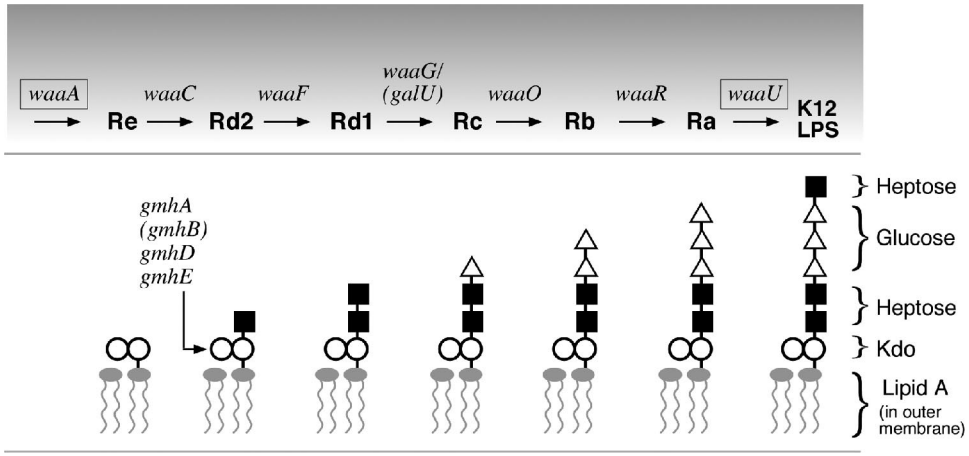

Fig. 3. Cartoon of the LPS structures on *E. coli* mutants. The biosynthesis pathway of the core LPS formation is indicated above the structures. Boxed genes are not represented in the Keio collection. Parenthesized genes display partial phenotype. The nomenclature of the LPS chemotypes was reviewed by Raetz (28).

glucose to the two core heptoses, as is the case in *waaO* host, allows T7 to infect the host implies that the first glucose of the LPS core is a binding site for T7. However, when one or more glucose residues are attached to this glucose, the binding site is probably hindered, and therefore either the penultimate glucose or the terminal heptose of the K-12 LPS backbone or both become essential for T7 infection. We also conclude that the side chains (not shown in Fig. 3) of the LPS are not important for T7 recognition, because the genes catalyzing these steps were not identified in the screen. Indeed, WT T7 showed similar EOP when tested individually on *waaL*, *waaP*, *waaQ*, *waaS*, *waaY*, and *waaZ* hosts (lacking genes that catalyze LPS side-chain additions/modifications), as compared with its EOP on WT *E. coli*. The EOP of WT T7 on *waaB* host (lacking a gene that catalyzes LPS side-chain addition) was slightly reduced (≈0.6) relative to the EOP on WT *E. coli*, but the plaques had normal morphology. Although this study identifies the complete LPS genotype associated with T7 resistance, others have also isolated *E. coli* K-12 mutants resistant to T7 (26). They postulated that the LPS moieties that form the binding site for T7 are the inner core heptoses, based on differential recognition patterns of various phages. Indeed, the *waaF* mutant, having Rd2-type LPS with only one heptose protruding can support phage infection to some extent, with a relatively high EOP of ≈0.2, as compared with the other mutants. This finding explains why the heptoses might have been considered the binding site for T7 receptor. However, our study implies that other moieties of the LPS, namely the first glucose, and the terminal glucose/heptose may play a more significant role in receptor recognition.

Overall, we have identified one gene previously known to be essential for T7 growth and 10 additional genes essential for T7 phage growth. The findings can be used to construct phages that are independent of nonessential host genes and consequently more virulent to the host. One approach toward achieving this goal is to insert the *trxA* and *cmk* genes into the T7 genome, thus reducing T7 dependency on host genes. Indeed, *trxA* has been inserted into many strains of T7 and these phages grow independently of host *trxA* expression. In addition, we have successfully introduced a *cmk* gene into T7 to select for recombinant phages, which do not depend on *cmk* expression by the host. These results show that (*i*) *cmk* can be introduced to T7 and thus render the phages independent of host *cmk*, and (*ii*) *cmk* can be used as a marker to select for T7 encoding it, in a similar way that *trxA* has been used (quite efficiently, as described in *Materials and Methods*). The availability of a second genetic marker simplifies genetic manipulations, for example, in constructing double deletion T7 mutants.

Construction of T7 Phages That Are More Virulent Than WT T7. From the data presented above it is clear that resistance to infection by phage T7 is most likely to arise by mutations in one or more of the 10 genes in the pathway leading to synthesis of the T7 receptor, the LPS. Therefore, we were curious to see whether we could isolate more virulent T7 phages, more virulent in the sense that less host resistance develops, by selecting for phages that can infect *E. coli* having alterations in the LPS that render them resistant to infection. Following the selection procedure in *Materials and Methods* we first selected for T7 phages that could infect *E. coli* having the shortest LPS (Re LPS in Fig. 3). As shown in Table 3, the selected mutant, T7-Re, can infect a host with the Re LPS as efficiently as WT T7 can infect WT *E. coli*. Interestingly, T7-Re can still infect WT *E. coli* with high efficiency. In addition, it can now infect *E. coli* mutants having defects in the LPS that give rise to LPS Rd1 and at a lower efficiency also to Rb (*waaG* and *waaR*, respectively). Inasmuch as T7-Re remained unable to infect strains with the Rd2 and Rb LPS (*waaF* and *waaR*) we selected for T7-Re phages that could grow on a Rb LPS (*waaR* host). The resulting mutant phage, T7-ReRb, gained the ability to infect *waaR* hosts and surprisingly retained its ability to grow on all of the other LPS mutants that T7-Re could infect (Table 3). In fact, its ability to grow on *E. coli waaR* was enhanced. The final selection was for a T7-ReRb phage that could grow on the one remaining LPS Rd2 mutant, *E. coli waaF* in the presence of K12 and Ra-purified LPS (to reduce the possibility of reversion to the WT). Again the selection yielded a phage, T7-ReRd2Rb, that retained its previous LPS phenotype but could also grow on *E. coli waaF* (Table 3). In this manner, as shown in Table 3, we constructed a mutant T7 phage that is essentially independent of the LPS receptor.

Table 3. Plating efficiency of the indicated phage on LPS mutants

	E. coli strain (LPS structure)					
Phage	K12 (K12)	*waaR* (Rb)	*waaO* (Rc)	*waaG* (Rd1)	*waaF* (Rd2)	*waaC* (Re)
WT T7	+++	−	+++	−	+	−
T7-Re	+++	+	+++	+++	−	+++
T7-ReRb	+++	+++	+++	+++	−	+++
T7-ReRd2Rb	+++	+++	+++*	+++	+++	++

EOP of indicated phage on indicated host, relative to its EOP on WT *E. coli*: +++ indicates 0.7 ≤ EOP; ++ indicates 0.3 ≤ EOP < 0.7; + indicates 0.1 ≤ EOP < 0.3; − indicates EOP < 0.1.
*EOP > 10.

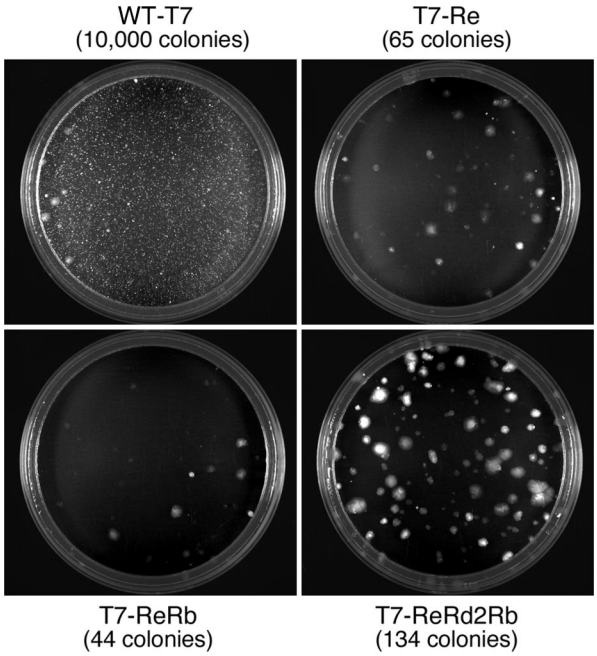

Fig. 4. Resistance to WT T7 and T7-Re, T7-ReRb, and T7-ReRd2Rb. *E. coli* cells (5×10^8 total in 3 ml of soft agar) were infected by the indicated phage at a MOI of 0.5 and overlaid on plates. After overnight incubation, resistant colonies could be visualized on the plates. The number of resistant colonies arising on each plate is indicated in parentheses.

We determined the frequency of host resistance to WT T7, T7-Re, T7-ReRb, and T7-ReRd2Rb. These four phages were used to individually infect 2×10^8 or 10^7 WT *E. coli* at a multiplicity of infection (MOI) of 0.5 and 10, respectively. As expected, host resistance to WT T7 was significantly higher (≈10,000 and ≈800 resistant colonies using MOI of 0.5 and 10, respectively) than host resistance to T7-Re (65 and 8 resistant colonies using MOI of 0.5 and 10, respectively), T7-ReRb (44 and 7 resistant colonies using MOI of 0.5 and 10, respectively), and T7-ReRd2Rb (134 and 31 resistant colonies using MOI of 0.5 and 10, respectively). Thus, T7-Re and T7-ReRb are at least 100-fold more virulent than WT T7. The remaining resistant cells most likely contain mutations in genes not related to the LPS biosynthesis pathway. Fig. 4 shows the colonies arising after infection by the indicated phage at an MOI of 0.5. The colonies on the WT T7 plate are mostly small and not mucoid, whereas the colonies on the mutant phage are mostly large and mucoid. This result suggests that up-regulation of the *rcsA* gene (Table 1), or other mutations leading to the increased capsule formation, can be the factor bestowing phage resistance in these colonies.

In this article, we highlight the importance of developing screens to identify host–virus interactions and their subsequent possible use for boosting phage therapy. The proofs of principles presented here for the relatively simple T7 and its host *E. coli* might pave the way toward similar use with other phages. When a collection of knockout mice becomes available (27), the principles presented here could be applied to identify host genes essential to eukaryotic viruses. The identity of genes that are essential for virus replication could lead to strategies that prevent or hinder viral growth.

Materials and Methods

Materials. LB broth, LB agar, granulated agar, and kanamycin were purchased from EMD (San Diego, CA). Chloramphenicol was purchased from American Bioanalytical (Natick, MA).

Bacterial Strains and Phage. Bacterial strains were provided by Hirotada Mori (Nara Institute of Science and Technology, Nara, Japan). The Keio collection was constructed on *E. coli* K-12 BW25113: *lacI*^{q}, *rrnBT14*, Δ*lacZWJ16*, *hsdR514*, Δ*araBADAH33*, and Δ*rhaBADLD78* (1). The ASKA collection was constructed by using *E. coli* K-12 AG1 (Stratagene, La Jolla, CA), which is a derivative of DH1: *recA1*, *endA1*, *gyrA96*, *thi-1*, *hsdR17* ($r^{K-}m^{K+}$), *supE44*, and *relA1*. Bacteria and phage were grown at 37°C, unless indicated otherwise.

All T7 phage used were from either our laboratory collection or William F. Studier's collection (Brookhaven National Laboratory, Upton, NY). The strains used for screening the ASKA library and the Keio collection are listed in Table 4.

Screening Procedure. The lowest phage concentration that eliminated replica-plated T7-sensitive cells but not *trxA* cells was used for the ASKA and Keio screens [D104/LG37: 1.2×10^4 pfu/ml; HS33: 3×10^4 pfu/ml; LG30: 7×10^4 pfu/ml (in soft agar)]. The optimal IPTG concentration for use in the ASKA screen was determined as follows: ≈50% of the colonies in the ASKA library are not viable at IPTG concentrations >1 mM (2). At an IPTG concentration of 0.1 mM, >95% of the colonies grow normally (in the absence of T7). *E. coli*/*dgt* served as a control strain for determining the optimal concentration of IPTG that would not be toxic to the cells while allowing for sufficient protein production. When *dgt* is overexpressed, it renders the bacteria resistant to T7 phage lacking gene *1.2* (4). Forty colonies of T7-sensitive bacteria and eight colonies of *E. coli*/*dgt* were replica-plated on plates containing phages and different IPTG concentrations. When no IPTG was present, all colonies were eliminated by the phage. When 0.1 mM IPTG was added to the 3 ml of soft agar, the 40 T7-sensitive colonies were eliminated, whereas the 8 *dgt*-harboring colonies survived. At concentrations >0.5 mM IPTG, all colonies were eliminated, because of either T7 sensitivity or overexpression toxicity. We therefore used 0.1 mM IPTG (in soft agar) for the ASKA library screens. In addition, we screened with 1 mM IPTG to identify nontoxic proteins that confer T7 resistance only when highly overexpressed.

The ASKA collection was replica-plated directly from frozen glycerol stocks on large LB-chloramphenicol-agar dishes (240 × 240 mm) overlaid with soft agar containing phages and IPTG. The plates were incubated at 37°C for 20 h. Surviving colonies were taken from their original glycerol stock for plasmid extraction, and the plasmids were retransformed into fresh *E. coli* AG1. The transformed colonies were retested under similar conditions. Colonies that consistently showed resistance to T7 growth were chosen for further analysis. Only colonies that had a continuous lawn on the replica-plated spot were chosen; bacteria that produced spots

Table 4. Strains used for screening the ASKA library and Keio collection

T7 phage strain	Deleted segment*	Deleted genes
D104/LG37	579–2736; 5847–7762	*0.3*, *0.4*, *0.5*, *0.6*, *0.7*, *1.1*, *1.2*, *1.3*, *1.4*
LG30	6575–8434	*1.3*, *1.4*, *1.5*, *1.6*, (*1.7* truncated at 5′ end)
HS33	13279–14284	*4.3*, *4.5*, *4.7*

*Base pair numbers correspond to T7 genome, GenBank accession no. NC_001604.

containing only one or several colonies proved to be not reproducible and were treated as false positives.

The Keio collection was replica-plated on large LB-kanamycin-agar Petri dishes overlaid with soft agar containing phage. The plates were incubated at 37°C for 20 h. Surviving colonies were retested by using an independent gene-replacement mutant colony provided in the Keio collection. Colonies that consistently showed resistance to T7 growth were chosen for further analysis. As with the ASKA collection screen, only bacteria that showed a continuous lawn of bacteria on the replica-plated spot proved to be actual T7-resistant colonies.

EOP Assays. Selected colonies that were scored positive in the ASKA screen were grown overnight, and 1 ml of the culture was mixed with ≈100 pfu WT T7 in soft agar. The mixture was overlaid on LB plates, and after 15 h plaques were counted. The pfu were normalized to the pfu obtained on a mock-transformed *E. coli* (*E. coli* with a plasmid expressing a gene that does not affect T7 growth, *thrS*).

A similar assay was carried out to determine the EOP by the colonies that scored positive in the Keio screen. In this case, the pfu counts were normalized to pfu counts obtained on a mock knockout strain (*E. coli* with a kanamycin insertion in a gene that does not affect T7 growth, *yhdQ*).

Complementation Assays. To validate the results obtained with the Keio collection, plasmids encoding the knocked-out genes were extracted from colonies in the ASKA library and transformed to their respective strains in the Keio collection. These transformed strains were checked for their EOP as described above.

Kinetic Lysis Assays. Midlog phase bacteria expressing either *udk* or the control *thrS* were grown in the presence of 1 mM IPTG for 45 min. A total of 180 μl of the cell suspension was then dispensed to wells in a 96-well plate. Twenty microliters of WT T7 and T7 phage mutants was added to the cell suspension at an MOI of 10^{-7} to 10^{-4} (similar MOI of each phage was used on the *udk*- and *thrS*-overexpressing hosts). The plate was incubated for 15 h in a Spectramax M5 spectrophotometer (Molecular Devices, Sunnyvale, CA) at 37°C, and every 5 min the plate was shaken and OD_{600} was measured. Differences similar to those shown in Fig. 2 were also observed when using higher MOIs.

Using *cmk* as a Selection Marker for *cmk*-Encoding T7 Phage. Δ*5*::*cmk* phage was generated by recombination between WT T7 phage and a pGp5-*cmk* plasmid encoding the *cmk* gene flanked by sequences upstream (400 bp) and downstream (200 bp) of gene *5*. The pGp5-*cmk* was generated by using multistep PCR and a TOPO cloning kit (Invitrogen, Carlsbad, CA). Liquid culture of DH5α cells transformed with pGp5-*cmk* plasmid at an OD_{600} ≈0.3 was infected with T7 WT phage at a MOI of 0.1 and grown until complete lysis. The lysate was plated on DH5α cells transformed with pGP-5 plasmid, encoding WT gp5. Forty-eight large plaques were transferred to a microtiter plate and replica-plated on *E. coli* strains DH5α, K-12Δ*cmk*, and K-12Δ*cmk*/pGP-5. All 48 plaques exhibited a phenotype, expected for Δ*5*::*cmk* T7 phage, i.e., negative for growth on DH5α and K-12Δ*cmk* (because of lack of gene *5*), and positive for growth on K-12Δ*cmk*/pGP-5, where gene *5* is supplied from the pGP-5 plasmid and *cmk* is supplied by the recombinant phage. Three plaques were randomly picked and the gene replacement was confirmed by direct sequencing of the genomic DNA from the recombinant phage.

Selection and Sequencing of Phage Mutants that Infect T7-Resistant Colonies. To obtain T7-Re, T7-ReRb, and T7-ReRd2Rb, selection of plaques that form on strains that have truncated LPS was sequentially carried out. T7-ReRb was sequenced from base pairs 17649–39937. The identified mutations map to genes *11*, *12*, and *17*: gp11-M6V, gp12-D181G, gp12-P694T, gp17-N501H, and gp17-R542H. The other two mutants and the WT T7 were sequenced in genes *11*, *12*, and *17* only. T7-Re contains the following substitutions: gp11-M6V, gp12-D181G, and gp17-N501H. T7-ReRd2Rb contains the following substitutions: gp11-M6V, gp12-D181G, gp12-P694T, gp17-N501H, gp17-R542H, and gp17-V544A. The WT T7 used in our laboratory is identical in these three genes to the published sequence (GenBank accession no. NC_001604) in all encoded residues except gp12-S694P.

Determining the Number of *E. coli* Colonies that Survive T7 Infection. The virulence of T7-Re, T7-ReRb, T7-ReRd2Rb, and WT T7 phage was determined as follows. Soft agar containing 5×10^8 cfu or 10^7 cfu of WT *E. coli* was mixed with each phage at an MOI of 0.5 and 10. The suspension was overlaid on LB plate and incubated at 30°C. The next day, surviving bacteria were counted.

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